

Utilization and interconversions of purine derivatives in the fission yeast *Schizosaccharomyces pombe*

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SUMMARY

In the fission yeast *Schizosaccharomyces pombe*, growth responses of mutants strains in the de novo purine synthesis pathway, in the purine interconversion system, and of various double mutants, have been studied upon different purine-supplemented media. The results show that exogenous purine utilization as nucleotide source is based exclusively upon pyrophosphorylation of purine bases, and they make it possible to identify most of the enzymic steps acting, in this organism, upon a purine ring to give another purine ring.

A scheme of the interconversion system in *Schizosaccharomyces pombe* is given.

1. INTRODUCTION

Besides the classical interest linked to the study of cellular metabolism and of its regulation, the growing economic importance of purine nucleotides, mainly as flavouring agents, but also because of their medical and nutritional effects, has given a new impetus to research in the purine metabolism field. A characteristic feature of purine metabolism is the fact that purine compounds are readily interconverted to each other by living cells; but though the de novo biosynthesis pathway of adenylic and guanylic acids seems to be identical throughout all living organisms (for a review see, for instance, Moat & Friedman, 1960), the interconversion mechanisms vary widely with varying organisms (for a review see for instance Balis, 1968).

This report is an attempt to elucidate the purine utilization and interconversion pathways in a given micro-organism, namely the fission yeast *Schizosaccharomyces pombe*, by the use of simple genetical and physiological methods.

2. MATERIALS AND METHODS

All chemicals used in this study are commercially available; labelled purine bases were obtained from the Commissariat à l'Énergie Atomique.

Minimal medium is a synthetic mineral medium supplemented with 5 g/l glucose. Utilisation of purine compounds as sole source of nitrogen was tested by removing the ammonium sulphate, which is the only inorganic nitrogen

source of the minimal medium, and replacing it by 200 mg/l of the purine compound under study. Low nitrogen medium contained 80 mg/l ammonium sulphate instead of 5 g/l.

Growth-tests and measurement of uptake have been described elsewhere (Pourquié, 1970).

(i) *Identification of excreted products*

Excretion was first detected in cross-feeding tests by the growth of an indicator auxotrophic strain included in agar solidified minimal medium. Excreted products were further identified by adsorption of supernatant from liquid cultures upon active charcoal, followed by acetone (80 %):water (20 %) desorption; desorbed products were then passed through a DEAE Sephadex column eluted by rising concentrations of tris-buffer; optically active peaks were concentrated and further characterized by their u.v. absorption spectra at different pHs and thin layer chromatography behaviour.

(ii) *Strains*

The wild-type strain is the 972 h⁻.

Most of the mutant strains have already been reported by Leupold (1955), Heslot (1960), Heslot, Nagy & Whitehead (1966) and De Groodt *et al.* (1969); they were obtained following mutagenic treatment with nitrosomethyl-urethane (NMU) of wild-type cell suspensions and plating on appropriate media. NMU was added to cell suspensions to give a final concentration of 10⁻⁴ (v/v) and incubation of the treated suspension in a shaking water-bath at 25 °C for 30 min resulted in about 50 % survival.

For the purpose of this work, purine-requiring mutants have been grouped in four classes corresponding to the position of the enzymic block in the pathway and the resulting qualitative purine requirement. These classes are:

hyp mutants; these mutants have an enzymic block before the CAIR → SAICAR* step; they can use hypoxanthine as well as adenine for growth.

gua mutants; these mutants have an enzymic block in one of the two steps leading from IMP to GMP; they can be grown upon guanine or 2,6-diaminopurine.

ade mutants; these mutants have an enzymic block in one of the two steps leading from IMP to AMP; they show a specific adenine requirement. It should be noted that the enzyme under the control of *ade 8* locus, is implicated in the catalysis of two different steps of purine nucleotide biosynthesis: conversion of SAICAR to AICAR and conversion of S-AMP to AMP.

ade 10 mutants; these mutants have an enzymic block in the conversion of AICAR to IMP; they show an additional histidine requirement; one of the explanations for this histidine requirement is that AICAR accumulated by these strains inhibits the first enzyme of histidine biosynthesis (Whitehead, 1966). *ade 10* mutants are repaired by hypoxanthine as well as by adenine. Strains

* Abbreviations: AMP, Adenylic acid; IMP, inosinic acid; GMP, guanylic acid; XMP, xanthylic acid; AICAR, amino imidazole carboxamide ribotide; APRT, adenine phosphoribosyl transferase; SAICAR, succinyl-AICAR; SAMP, succinyl-AMP.

lacking one of the two phosphoribosyltransferase activities have also been employed; *pur 1* is devoid of hypoxanthine-guanine-phosphoribosyltransferase (E.C. 2.4.2.8) actively, *dap 1* is devoid of adenine-phosphoribosyl transferase (E.C. 2.4.2.7) activity; *pur 1* was selected as resistant to 8-aza-2-thioxanthine (De Groot *et al.* 1969) and *dap 1* was selected as resistant to high concentrations of 2,6-diaminopurine (De Groot *et al.* 1969).

The growth of *gua 1* strains upon minimal medium supplemented with 20 mg/l guanine is completely inhibited by the addition of 20 mg/l adenine; after mutagenesis of *gua 1* strains, *ins 1* mutation was selected as giving resistance up to 40 mg/l adenine.

Dea 1 mutation was obtained from a *gua 1 ins 1* strain which was not able to use 2,6-diaminopurine as GMP source while it could still use guanine.

aza 1 mutation has been shown to be a feedback resistance mutation (Heslot *et al.* 1966; Nagy, 1970); it is closely linked to the *hyp 4* locus and is phenotypically expressed by an over-production of IMP precursors and an excretion of inosine and hypoxanthine.

Apart from *hyp 4* and *aza 1* no clustering or close linkage of the genes quoted in this work has been observed.

3. RESULTS

(i) Growth test results

The de novo purine biosynthesis pathway in *Schizosaccharomyces pombe* and the localization of the enzymic blocks as known from previous studies are shown in Fig. 1.

Table 1 gives the growth-test responses of the different strains upon different supplemented media. Lethality of *ade dap 1* and *gua pur 1* double mutants has been deduced from the inability of the spores of this genotype to germinate upon

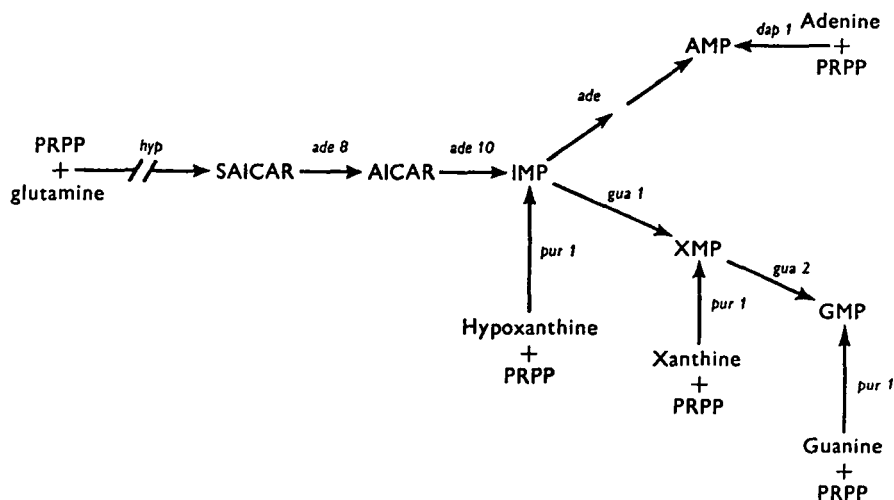


Fig. 1. Purine mononucleotide biosynthesis in *Schizosaccharomyces pombe*.

any supplemented medium (the genotype of the spores was inferred from the genotypes of the other spores of the tetrads resulting from crosses between *ade* and *dap 1* parent strains and *gua* and *pur 1* parent strains).

Table 1. *Growth responses of the different strains to different purine supplementations (+, growth; 0, absence of growth)*

(The concentration of the purine compounds was 15 micromoles.)

	Hypoxanthine	Inosine	IMP	Adenine	Adenosine	AMP	Guanine	Guanosine	GMP	Xanthine	Xanthosine	XMP	6-Chloropurine	2,6-diaminopurine
<i>hyp</i>	+	+	+	+	+	+	0	0	0	0	0	0	+	0
<i>hyp pur 1</i>	0	0	0	+	+	+	0	0	0	0	0	0	0	0
<i>hyp dap 1</i>	+	+	+	+	+	+	0	0	0	0	0	0	+	0
<i>ade</i>	0	0	0	+	+	+	0	0	0	0	0	0	0	0
<i>ade pur 1</i>	0	0	0	+	+	+	0	0	0	0	0	0	0	0
<i>ade dap 1</i>							Lethal							
<i>gua 1</i>	0	0	0	0	0	0	+	+	+	+	+	+	0	+
<i>gua 1 dap 1</i>	0	0	0	0	0	0	+	+	+	+	+	+	0	+
<i>gua 1 pur 1</i>							Lethal							
<i>gua 2</i>	0	0	0	0	0	0	+	+	+	0	0	0	0	+
<i>gua 2 dap 1</i>	0	0	0	0	0	0	+	+	+	0	0	0	0	+
<i>gua 2 pur 1</i>							Lethal							
<i>gua 1 ins A</i>	0	0	0	0	0	0	+	0	0	+	+	+	0	+
<i>gua 2 ins A</i>	0	0	0	0	0	0	+	0	0	0	0	0	0	+

Table 1 shows that (1) *hyp* mutants grow on hypoxanthine (or adenine) and the corresponding nucleosides and nucleotides; (2) *ade* mutants require specifically adenine or the corresponding nucleoside and nucleotide; (3) *gua* mutants require guanine or the corresponding nucleoside and nucleotide; (4) *ade* and *hyp* mutants do not require and are not repaired by guanine or xanthine; (5) *gua* mutants do not require and are not repaired by hypoxanthine (or adenine) and their derivatives; (6) 6-chloropurine can be used by *hyp* mutants, and 2,6-diaminopurine by *gua* mutants.

Observations (1)–(5) are readily understood by looking at the metabolic pathways on Fig. 1.

(ii) *Entry of purine compounds*

(a) *Entry of purine bases*

Direct evidence has been given regarding the active transport of adenine and guanine into the cells of *Schizosaccharomyces pombe* (Cummins & Mitchison, 1967; Pourquié, 1970). We may reasonably presume that hypoxanthine, xanthine, and other purine bases are handled in the same way and enter the cells in their intact form.

(b) Entry of purine nucleotides

When added to MM medium, purine mononucleotides support the growth of purine-requiring strains as well as their corresponding bases do (see Table 1). The question is whether they enter the cells in their intact form or not. The double mutants carrying both a *pur 1* and an *hyp* mutation are unable to synthesize IMP either de novo or from preformed hypoxanthine. If IMP entered the cells in its nucleotide form, it would support the growth of such double mutants. In fact it does not, so we conclude that IMP cannot enter the cells (at least in quantities sufficient to support growth); and we assume that since IMP supports the growth of *hyp* strains, it is degraded outside the cells into metabolites able both to enter the cells and to fulfill the growth requirement; that is, presumably inosine or hypoxanthine. The same argument applies for AMP and GMP.

(c) Entry of purine nucleosides

In general, nucleosides support the growth of purine-requiring mutants as well as their corresponding bases do. It has been shown (Cummins & Mitchison, 1967; Pourquié, 1970) that nucleosides do not compete with adenine and guanine

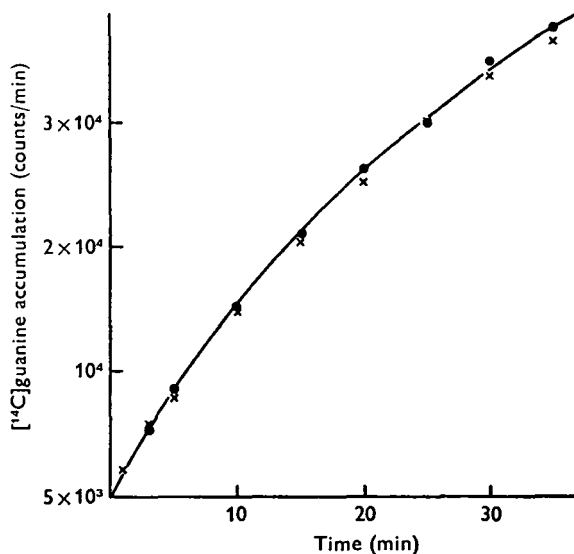


Fig. 2. Time course of guanine uptake. At zero time, 2 mg/l. ¹⁴C-labelled guanine was added to the medium; 2 ml aliquots of the cell suspension were removed at intervals and the amount of radioactivity retained by the cells was measured. ●, No guanosine present; ×, 100 mg/l. unlabelled guanosine added at the same time as the labelled guanine.

uptake sites; moreover, large quantities of unlabelled guanosine (100 mg/l.) in the external medium do not affect the rate of entry of small quantities of labelled guanine (2 mg/l.) over a 35 min period (Fig. 2), which should be the case if guanosine were transformed into guanine outside the cells. We may thus believe that

nucleosides are little if at all transformed outside the cells, and enter the cells through uptake sites different from those of the bases.

(iii) *Utilization and interconversion of guanine, guanosine, and GMP, and of xanthine, xanthosine and XMP*

Since exogenous guanine, guanosine, and GMP support the growth of *gua* strains, there must thus be a pathway leading from these exogenous compounds to endogenous GMP. We have seen above that GMP could be formed in a single enzymic step from guanine and PRPP. Guanosine cannot be converted in one step into GMP, otherwise *gua pur 1* double mutants would be able to use guanosine

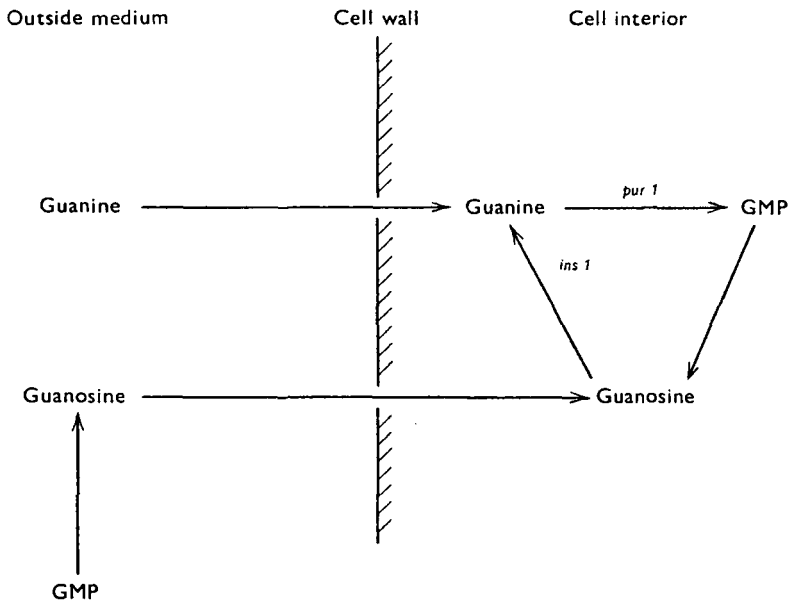


Fig. 3. Utilization and interconversion of exogenous guanine, guanosine and GMP.

as GMP source. We can be sure that growth of *gua* strains upon guanosine-supplemented media occurs through a degradation of guanosine into guanine which in turn is converted into GMP, since any other alteration of guanosine would give compounds which could not be used by *gua* strains, especially by *gua 2*. This enzymic degradation of guanosine into guanine, is probably affected by the mutation called *ins 1*: indeed, *gua ins 1* double mutants are unable to grow upon guanosine while they still grow upon guanine, *ins 1* strains crossfeed both *gua 1* and *gua 2* strains and it could be shown that guanosine but not guanine was excreted by these strains, and finally the *ins 1* strain can use guanosine as nitrogen source, indicating that the *ins 1* mutation does not affect guanosine uptake.

We then conclude that pyrophosphorylation of guanine by the enzyme phosphoribosyl-transferase is an obligatory step in the utilization of exogenous

guanine, guanosine, and GMP, which have first to be transformed into endogenous guanine in order to serve as GMP source. This most likely scheme of interconversions between guanine, guanosine, and GMP is indicated in Fig. 3. Xanthine, xanthosine and XMP behave towards *gua 1* mutants in the same manner as guanine, guanosine, and GMP do towards *gua 1* and *gua 2* mutants; however, the *gua 1 ins 1* strain utilizes as GMP source xanthosine as well as xanthine or guanine. This probably implies that a specific enzyme, not coded by *ins 1*, is responsible for the conversion of xanthosine to xanthine.

(iv) *Utilization and interconversion of hypoxanthine, inosine, and IMP and of adenine, adenosine and AMP*

The same arguments as those presented above for the formation of GMP can be applied to the formation of IMP, using *hyp* and *hyp pur 1* strains, and to the formation of AMP, using *ade* and *ade dap 1* strains. However, no mutation analogous to *ins 1* is yet available (*ins 1* affects only guanosine utilization by *gua ins 1* double mutants but not inosine utilisation by *hyp ins 1* double mutants). Inosine is excreted by any strain which accumulates or over-produces IMP, such as *ade 2* and *gua 1*, which accumulate IMP because of their genetic block, and *aza 1* which overproduces IMP because of its feedback insensitivity (Heslot *et al.* 1966).

Adenosine is very poorly used by *ade* strains; growth responses which are usually visible after 3 days incubation in the droplet tests (see Materials and Methods), cannot be obtained before 5 or 6 days incubation upon adenosine-supplemented minimal medium. Adenosine is nevertheless not used at all as AMP source by *ade dap 1* strains; Cummins & Mitchison (1967) did not record any adenosine pool formation after adenine feeding but, instead, they report the formation of an inosine pool which they consider as a purine storage pool. Adenosine is thus probably deaminated to inosine rather than degraded to adenine; this deamination would account for the poorer growth of *ade* mutants upon adenosine-supplemented minimal medium, compared to the growth of *hyp* strains upon inosine-supplemented minimal medium.

The scheme of relationships between hypoxanthine and adenine base, nucleoside, and nucleotide is thus probably the same as the one for guanine derivatives.

(v) *Transformation of the purine ring*

Four main kinds of interconversion acting in a single enzymic step on a given purine ring to yield another purine ring are known in the literature; they are: hydrolysis of a C-6 substituent yielding, for instance, hypoxanthine from adenine; hydrolysis of a C-2 substituent yielding, for instance, xanthine from guanine; oxydation at the C-2 and C-8 positions yielding, for instance, xanthine and uric acid from hypoxanthine and xanthine; reduction of the C-2 position yielding, for instance, IMP from GMP. In *Schizosaccharomyces pombe*, GMP reductase, which in some bacterial species allows the conversion of GMP into IMP and hence the growth of adenine-requiring mutants upon guanine supplemented media, has no

physiological role since *hyp* strains cannot grow upon any guanine- or guanosine-supplemented media. On the other hand, the three other types of reaction are likely to occur.

(a) *Hydrolysis of C-6 substituent*

The following facts argue for the existence of an *in vivo* hydrolysis of C-6 substituents of the purine ring: 2,6-diaminopurine supports the growth of *gua* strains as well as guanine or guanosine do; such a situation has been reported for other organisms (see, for instance, Kalle & Gots, 1961), and the simplest explanation for this fact is to suppose that a hydrolysis occurs at the C-6 position yielding directly a guanine ring from a 2,6-diaminopurine ring. This hydrolysis

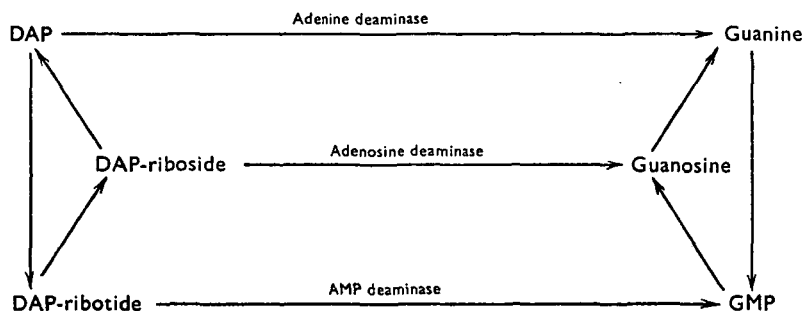


Fig. 4. *A priori* possibilities of getting GMP from 2,6-diamino-purine (DAP).

can also affect other substituents than amino substituents; this is best exemplified by the fact that *hyp* strains can use as nucleotide sources various C-6 substituted purines such as 6-chloropurine or 6-mercaptapurine. Adenine, adenosine and AMP aminohydrolases have been reported in the literature and are known to catalyse these kinds of hydrolysis, but their distribution varies widely from one organism to one another. The question was then to know what was the situation in *Schizosaccharomyces pombe*; as we have seen that adenine, adenosine, and AMP could be readily interconverted, hydrolysis in C-6 may, *a priori*, occur either upon one, two, or the three compounds (Fig. 4).

Evidence for an adenine aminohydrolase activity. *Hyp dap* strains show a purine requirement but are unable to convert directly adenine into AMP through APRT activity; however, these double mutants can use adenine as nucleotide source. This is an indication that adenine can be deaminated to hypoxanthine which is in turn converted into IMP. Moreover adenine amino-hydrolase activity has been detected *in vitro* tests upon crude extracts of *Schizosaccharomyces pombe* (Abbondandolo *et al.* 1970).

Evidence for an absence of AMP amino-hydrolase activity. AMP amino-hydrolase can be thought to be absent in this yeast; two kinds of argument lead to this conclusion. C-6 substituted purines which are believed to be converted into nucleotide analogs, do not support the growth of *hyp pur 1* double mutants; as these strains cannot grow upon hypoxanthine or inosine, this result shows that

no enzyme is able to hydrolyse C-6 substituents of a mononucleotide analog. On the other hand, the growth of *hyp pur 1* on adenine is inhibited by high levels of histidine in the growth medium; histidine inhibits its own synthesis and therefore the production of AICAR, the immediate and only precursor of IMP for these strains (AICAR is a by-product of the fifth step of the histidine pathway); if IMP production was due to direct deamination of AMP, it would not be expected to be sensitive to histidine inhibition (histidine has been shown by Whitehead, Nagy & Heslot (1966) to inhibit the first enzyme of its own biosynthesis in *Schizosaccharomyces pombe*). A further proof of the absence of deamination of AMP will be given below.

Evidence for an adenosine amino-hydrolase activity. If adenosine amino-hydrolase was not active, it would be easy to select mutants impaired in adenine amino-hydrolase activity, since this activity would be absolutely necessary to strains such as *gua* when grown upon 2,6-diaminopurine but not when grown upon guanine; such mutants would use guanine but no more 2,6-diaminopurine. In fact, we could not succeed in isolating such mutants. The main reason for this failure is, we believe, the presence of an adenosine aminohydrolase, able to by-pass a block in adenine amino-hydrolase activity. A mutation affecting adenine amino-hydrolase activity could be obtained by using instead of *gua* strains a *gua ins 1* strain. In this strain, even if guanosine is produced from 2,6-diaminopurine, this guanosine cannot be used due to the *ins 1* mutation which blocks the guanosine \longrightarrow guanine step. We thus obtained mutants from *gua ins 1*, unable to use 2,6-diaminopurine but still able to use guanine. This new mutation was called *dea 2*. It has been separated from the *ins 1* and *gua* mutations. The double mutant *gua dea 2* is able to grow upon 2,6-diaminopurine, and strains bearing only the *dea 2* mutation do not show any peculiar phenotype. It was further established that cell-free extracts of *dea 2* strains lacked amino-hydrolase activity (De Groot *et al.* 1969). The growth of *gua dea 2* strains upon 2,6-diaminopurine, in spite of an absence of adenine-amino hydrolase, clearly shows that adenosine amino-hydrolase is present and that the step 2,6-diaminopurine \longrightarrow guanine can be replaced by the pathway shown below (where DAP stands for 2,6-diaminopurine)

DAP \longrightarrow DAP mononucleotide \longrightarrow DAP riboside \longrightarrow guanosine \longrightarrow guanine.

(b) *Hydrolysis of the C-2 substituent*

Hydrolysis of the C-2 substituent of guanine certainly occurs in *Schizosaccharomyces pombe*, since *gua 1* strains are able to use 2-thioxanthine as GMP source while *gua 2* strains are not. However, no argument allows us to decide whether this hydrolysis occurs upon the base, the nucleoside, or the nucleotide.

(c) *Oxidation at the C-2 and C-8 positions*

Oxidation at the C-2 and C-8 positions of hypoxanthine and xanthine is usually brought about by either a xanthine oxidase (Dixon & Webb, 1964) or a xanthine

dehydrogenase (see, for instance, Darlington, Scazzocchio & Pateman, 1965). If the production of xanthine from hypoxanthine were of some importance in the synthesis of XMP, *gua 1* strains would not show any purine requirement; *gua 1* strains grow very poorly upon minimal medium. This leakiness could be accounted for by the action of xanthine oxidase upon hypoxanthine, since no IMP-dehydrogenase activity could be detected in extracts of *gua 1* strains. Xanthine oxidase is confidently thought to be the first enzyme of the purine degradation pathway; this pathway exists in *Schizosaccharomyces pombe*, which can use as sole nitrogen

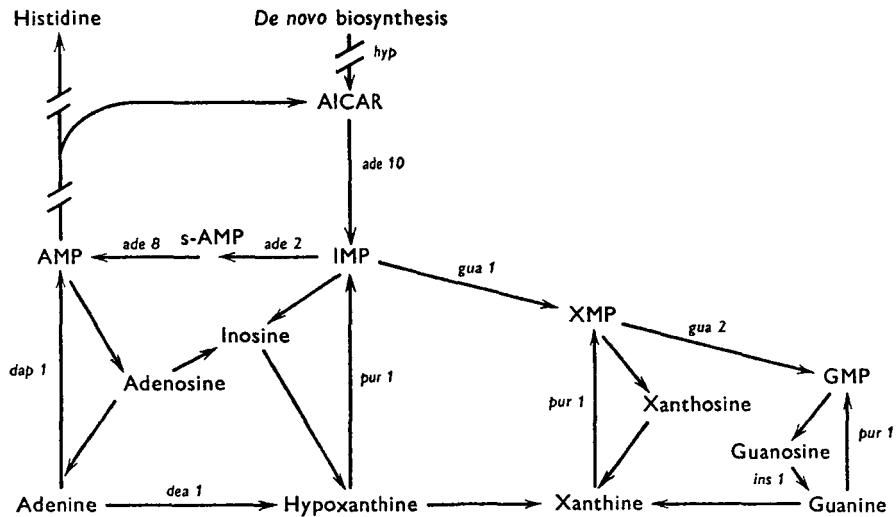


Fig. 5. General scheme of interconversion of purine derivatives in *Schizosaccharomyces pombe*.

source any natural purine compound, and particularly xanthine and hypoxanthine which do not possess any amino substituent; uric acid and allantoin are also used as nitrogen source. We believe, then, that xanthine oxidase is present and active in *S. pombe*, but that the product of its action upon hypoxanthine is mainly uric acid, xanthine being only a transient intermediate.

4. DISCUSSION

Most of the arguments presented in this report are based upon the growth responses of strains lacking one of the two purine phosphoribosyl-transferase; they imply that these strains are able to take up the various compounds tested. If they were not, these arguments would not stand. Berlin & Stadtman (1966) have presented evidence in another organism, *Bacillus subtilis*, for a direct participation of phosphoribosyl-transferases in the uptake of purine bases; yet Grenson (1969) has shown that, in *Saccharomyces cerevisiae*, translocases and phospho-ribosyl-transferases for the pyrimidine system were independent enzymic steps; strains which had lost phosphoribosyl-transferase activity showed a much

lower uptake than the wild-type strain, but were able to grow on pyrimidine as sole nitrogen source. Our strain *pur 1* shows a much-reduced uptake of labelled purine compounds, but grows on any purine base or nucleoside as sole nitrogen source.

The reduced uptake could be explained by an accumulation inside the cell of a metabolite controlling uptake rate; in fact, the *pur 1* strain accumulates and excretes hypoxanthine and inosine; in the same way the *aza 1* strain which is feedback insensitive (Heslot *et al.* 1966) excretes and accumulates hypoxanthine and inosine, and also shows a high reduction in uptake rate and a normal growth on purine bases as sole nitrogen source.

The overall scheme of purine interconversion is shown in Fig. 5. Additional steps, if they exist, are not of physiological importance in the culture conditions used in this study. This scheme is simple enough but shows good efficiency in the utilization of biological purines and in the resistance towards toxic analogs. The relative importance of the different steps cannot be ascertained until studies on acid-soluble pools have been carried out.

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